Neural Development Under Conditions of Spaceflight

Authors

Meredith D. Temple, Maria J. Denslow, Kenneth S. Kosik, Oswald Steward

Experiment Team

Principal Investigator: **Kenneth S. Kosik**[1]

Co-Principal Investigator: **Oswald Steward**[2]

Co-Investigators: **Meredith D. Temple**[3]
Maria J. Denslow[4]

Technical Assistant: **Justin Harder**[1]

[1]Harvard Medical School, Brigham and Women's Hospital, Boston, USA
[2]Reeve-Irvine Research Center, University of California at Irvine, Irvine, USA
[3]National Institute of Biomedical Imaging and Bioengineering, Bethesda, USA
[4]Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, USA

ABSTRACT

One of the key tasks the developing brain must learn is how to navigate within the environment. This skill depends on the brain's ability to establish memories of places and things in the environment so that it can form "cognitive maps." Earth's gravity defines the plane of orientation of the spatial environment in which animals navigate, and cognitive maps are based on this plane of orientation. Given that experience during early development plays a key role in the development of other aspects of brain function, experience in a gravitational environment is likely to be essential for the proper organization of brain regions mediating learning and memory of spatial information. Since the hippocampus is the brain region responsible for cognitive mapping abilities, this study evaluated the development of hippocampal structure and function in rats that spent part of their early development in microgravity. Litters of male and female Sprague-Dawley rats were launched into space aboard the Space Shuttle *Columbia* on either postnatal day eight (P8) or 14 (P14) and remained in space for 16 days. Upon return to Earth, the rats were tested for their ability to remember spatial information and navigate using a variety of tests (the Morris water maze, a modified radial arm maze, and an open field apparatus). These rats were then tested physiologically to determine whether they exhibited normal synaptic plasticity in the hippocampus. In a separate group of rats (flight and controls), the hippocampus was analyzed using anatomical, molecular biological, and biochemical techniques immediately postlanding. There were remarkably few differences between the flight groups and their Earth-bound controls in either the navigation and spatial memory tasks or activity-induced synaptic plasticity. Microscopic and immunocytochemical analyses of the brain also did not reveal differences between flight animals and ground-based controls. These data suggest that, within the developmental window studied, microgravity has minimal long-term impact on cognitive mapping function and cellular substrates important for this function. Any differences due to development in microgravity were transient and returned to normal soon after return to Earth.

INTRODUCTION

The Neurolab mission provided a unique opportunity to evaluate how microgravity affects brain and cognitive development. An important aspect of brain function is the ability to form memories of the environment, referred to as "cognitive maps" (O'Keefe, 1978). The processing and recall of spatial information enabling cognitive mapping depends on a part of the brain called the hippocampus and its related structures (O'Keefe, 1971; O'Keefe, 1979; O'Keefe, 1978; O'Keefe, 1980). Forming mental maps is an essential part of our existence. Imagine what it would be like not to have the capability to go to the store and successfully return home. Indeed, it is this very fundamental ability that is lost in neurological disorders such as Alzheimer's disease.

Although the ability to successfully navigate one's environment can be lost for any number of reasons, its proper development depends on early experiences (Paylor, 1992). For example, experience in a complex ("enriched") environment during early development can improve later cognitive performance in a number of tasks (Paylor, 1992). Animals that experience complex environments during early development have higher brain weight, increased cortical thickness, and increases in the number of synapses on cortical neurons. This indicates that experience actually affects the development of neural circuitry (Ferchmin, 1970; Walsh, 1981; Green, 1983).

Earth's gravitational field defines the primary planes of orientation of the spatial environment in which animals navigate, and the cognitive maps we develop are based on this plane of orientation. For example, humans think of floors, walls, and ceilings and "map" elements of the environment based on this planar orientation. Experience in a gravitational environment is likely essential for the proper development of brain regions that mediate learning and memory of spatial information. Normal development not only requires experience, a concept referred to as activity-dependence, but it also requires that the experience occurs within a certain window of time during development. A well-known example is the child who may lack sight due to premature cataracts or who has diminished sight in one eye due to a misalignment of the eyes. If these problems are not surgically corrected within the first few years of life, the brain will develop abnormally. After a certain age, the critical period to fix these problems will pass and the visual problems will be permanent. Sight is required during development to establish a normal visual system.

What is not known is whether the absence of gravitational cues would affect the normal development of the spatial cognitive navigation system. Studies involving the recording of individual cells within the hippocampus reveal that certain cells, now referred to as "place cells," fire preferentially when an animal is located at a particular position in its environment (Knierim, 2000; also see science report by Knierim et al. in this publication). Data suggest that the pattern of activity of place cells represents the location of the animal relative to particular environmental cues and that these cells and their connections may be critical for forming mental maps. Without gravity, it is possible that these connections could be altered. In fact, a series of experiments was also conducted as part of the Neurolab mission to study how living in microgravity affects place cell firing (Knierim, 2000; see also science report by Knierim in this publication).

The Neurolab Space Shuttle mission (April 1998) provided an unprecedented opportunity to learn the extent to which animals depend on gravity for the normal development of neural systems that store and analyze spatial information. Until now, it has been impossible to assess whether the absence of gravity would affect the development of spatial memory function. Hence, the purpose of the present study was to determine whether development in an environment lacking gravity would alter rats' navigational abilities, cognitive performance, hippocampal anatomy, and hippocampal cellular function upon return to Earth.

Because the Neurolab mission provided a novel opportunity to study the role of gravity in neural development, it was difficult to hypothesize what the specific impact of maturing during spaceflight and without gravity would have on proper neurological development. The absence of gravity could represent an "enriching" experience similar to that of a "complex environment," by allowing animals to experience a multidimensional rather than a two-dimensional environment. Alternatively, development in an environment without gravity could be deleterious, depriving animals of experience with the planar environment that is defined by Earth's gravity.

Our team evaluated the anatomy, neurophysiology, and biochemistry of the hippocampus, and also performed behavioral tests that require a normally functioning hippocampus in rats that had developed in microgravity. The results were compared to their Earth-bound counterparts.

METHODS

Flight paradigm

Rats of two different ages were launched into space on board the Space Shuttle *Columbia*. One group was eight days old (postnatal day eight (P8)) at the time of launch, and the other group was 14 days old (P14). The younger rats had not yet opened their eyes. At this age, rats spend essentially all of their time near their mother (a rodent pre-toddler stage of development). The older animals were at a stage of development three to four days following eye opening. At this age, rats are beginning to venture away from their mothers to explore their local environment.

Prior to launch, the young rats were evenly distributed among the birthing mothers and were designated the experimental flight (FLT) groups. Sixty-four hours before the launch of the Orbiter *Columbia*, the cross-fostered litters were loaded into Research Animal Holding Facility (RAHF) cages (P8 litters) or animal enclosure modules (AEMs) (P14 litters) with each cage holding one mother and eight young. Both the RAHF and AEM cages provided water (lixit devices) and food (spring-loaded food bars). Modifications were made so that the water and food could be delivered to the animals on orbit without the aid of gravity. The same procedure (pooling, redistribution, and

loading of rats) was performed on the control groups (also P8 and P14) designated vivarium (VIV) and asynchronous ground control (AGC), respectively. The VIV groups lived in standard animal vivarium housing, while the AGC groups lived in housing identical to that aboard the Space Shuttle *Columbia*. The AGC and VIV groups for both litters were run on four-day and eight-day delays with their timeline running exactly parallel to the FLT group.

The flight lasted 16 days, during which time food and water consumption in each cage was monitored regularly. Because rats mature rapidly over this time interval, both groups of rats reached young adulthood in space. Approximately eight hours after landing, the animals were removed from the Orbiter, examined by a veterinarian, and distributed to the individual labs for study. All animal care and experimental procedures conformed to National Institutes of Health (NIH) guidelines and were approved by the Institutional Animal Care and Use Committees at NASA Ames Research Center, California, the University of Virginia, and/or Harvard University, Massachusetts.

Behavioral analysis of flight animals

Upon return to Earth (R+0), a total of 43 rats from FLT litters of both age groups (P8 and P14 at launch) and their respective control groups were tested behaviorally over a one-month period. Three behavioral testing paradigms were used. One task (the Morris water maze) evaluates a rat's ability to learn and remember the location of a submerged platform in a tank filled with opaque water. There were constant cues in the room to provide spatial information about the location of the submerged platform. This task could be likened to the navigation problems faced by rats in the wild that live in semiaquatic environments (an urban sewer for example). Rats were tested as they learned to find the hidden platform to assess learning ability, and they were retested on R+25 (postlanding) to determine whether they remembered the location of the platform. Initial testing began three days after landing (R+3) for the P14 litters and nine days postlanding (R+9) for the P8 litters. During the initial testing, animals had four trials per day (one from each start location) for four consecutive days.

Another task evaluated the rats' ability to navigate on an eight-arm radial maze baited with highly attractive food substances. This task assesses rats' foraging strategies and is similar to the problems faced by rats seeking food in the wild. The optimal foraging strategy is one in which places are visited without repetition—that is, to avoid recently visited sites where food has already been found and removed. Hence, this task assesses memory of places recently explored. Animals were tested for five (nonconsecutive) days (two trials/day) on R+16, 19, 22, 25, and 27. Finally, we also assessed the rats' exploratory behavior in an open field. This free exploration task provided information regarding whether the rats would recognize walls as barriers. In space, walls are not barriers; instead, they are simply alternative surfaces upon which to navigate. The ability to recognize a wall as a barrier is likely to require experience with that boundary. All rats received one trial per day on days R+2, 3, 4, 5, and 7.

Electrophysiological analysis of hippocampal function

At the end of the behavioral testing, 22 rats from the P8 litters were assessed neurophysiologically to evaluate a form of synaptic plasticity in the hippocampus that is thought to play a role in the memory of place (long-term potentiation (LTP)). For this experiment, rats were anesthetized, and stimulating and recording electrodes were placed to activate key hippocampal pathways. We focused on the pathway called the "perforant path," which carries information from the cerebral cortex to the hippocampus. This is the pathway in which LTP was first discovered. To explore the capacity for LTP, synaptic responses generated by the perforant pathway were evaluated before and after the delivery of patterns of stimulation that are optimal for inducing LTP. The key issue was whether the rats that developed in space had the same capacity for LTP as rats raised on Earth. The stimulation to induce LTP involved the delivery of three "bouts" of stimulation, in which each bout consisted of 10 eight-pulse trains delivered at 400 Hz.

Histological analyses of flight hippocampus

On the day of recovery from the Space Shuttle *Columbia* (R+0), the brains of six rats from the P8 FLT group were studied. The tissue was fixed by microwave irradiation for five minutes while submerged in P.L.P. (2% paraformaldehyde, l-lysine, sodium periodate). The brains were then post fixed in P.L.P. for 18 hours and transferred on R+1 to a 15% glycerol solution in phosphate-buffered saline to cryoprotect the tissue. The same harvesting procedure was completed for the VIV (n=6) and AGC (n=6) control groups on the four-day and eight-day delay, respectively. Forty-micron coronal sections were taken at the level of the hippocampus. The tissue was collected in serial sections so that each group of sections contained a representative section of the hippocampus at ~800μ intervals. One group of serial sections was stained with a Nissl stain to mark the cell bodies. The remaining serial sections were then immunocytochemically stained using a free-floating technique. Antibodies against the following markers were used: phosphorylated neurofilament (Sternberger Monoclonals Incorporated (SMI) (Lutherville, MD) #31), non-phosphorylated neurofilament (SMI #312), synaptophysin (Sigma #SVP 38) (Sigma Chemicals Pty Ltd., Perth, Western Australia) and SNAP-25 (SMI #81), mGluR1 (Upstate Biotechnology, Inc., Lake Placid, NY), GluR 2/3 (Upstate Biotechnology, Inc., Lake Placid, NY), mGluR5, NMDA R1 (Chemicon International, Inc., Temecula, CA), NMDA R2A/B (Chemicon), and microtubule associated protein (Kosik Lab 5F9). All immunocytochemical stainings were labeled with a Cy 3 secondary fluorescent antibody and imaged on a confocal microscope. Z-series images were taken at 40× and 63× where each image of the Z-series represents 0.50 (of the section). Images were then transferred to the IPLab Imaging System (IPLab, Stockholm, Sweden) software and analyzed. For neurofilament staining, the optimal level was determined and the surrounding frames were averaged. The composite frame was then thresholded to highlight the immunopositive areas of the section. The level of thresholding was set to equal the mean intensity of each region analyzed. The

immunopositive areas were then calculated as a percent area of the total measured region, and neurofilament densities were calculated.

RESULTS

Flight rats exhibit normal learning and memory in spatial tasks

Despite the fact that the FLT rats spent much of their early development in a weightless environment, they exhibited no lasting behavioral abnormalities. The FLT animals in both age groups (P8 and P14) exhibited essentially normal learning during the initial Morris water maze testing (Figure 1) and normal memory during the retest (Figure 2). The same was true of the radial arm maze (data not shown). Rats also exhibited normal exploratory behavior in the open field (including normal responses to walls) (data not shown).

The only exception involved the FLT group launched at 14 days of age. These rats began testing in the Morris water maze on the third day after landing (R+3) and took longer to find the hidden platform and swam faster during testing that day. However, by the second day of testing, the P14 FLT rats' performance was comparable to the control groups.

Flight rats, regardless of age, used different search strategies in the early phases of Morris water maze testing. By the third day of maze testing, both FLT groups were using the same strategy as control animals. For complete data analysis, see Temple et al. (Temple, 2002).

Flight rats exhibit a normal capacity for hippocampal synaptic plasticity

Long-term potentiation (LTP) is a form of synaptic plasticity that is thought to be important for the establishment of memories. LTP is seen when particular pathways are activated by brief high-frequency trains of stimuli (similar to the patterns of activity exhibited by neurons in the hippocampus and related structures). The standard paradigm for assessing LTP is to deliver single-pulse stimulation to the perforant path while recording synaptic responses from a structure called the dentate gyrus in the hippocampus. In this study, we first determined "baseline" response amplitude, and then delivered three bouts of high-frequency stimulation to induce LTP. P8 FLT rats exhibited similar baseline responses, and exhibited the same capacity for LTP as their respective controls (Figure 3). These results demonstrate that by one month after returning to Earth, animals that developed in space exhibited no detectable physiological abnormalities. It will be of considerable interest to determine whether

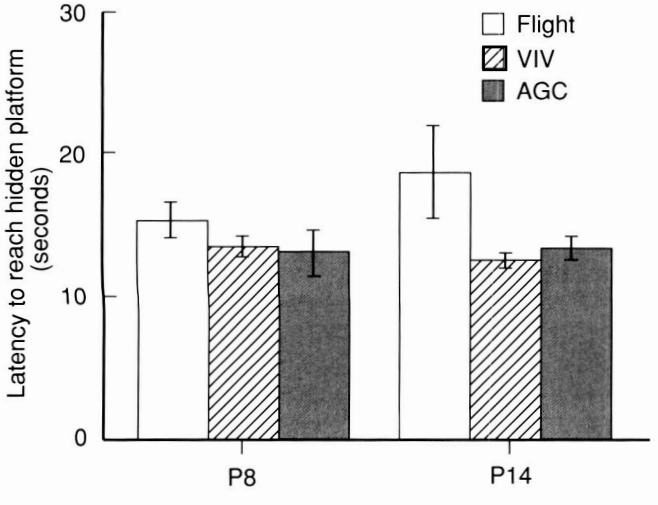


Figure 1. Cognitive performance was assessed using a hidden platform version of the Morris water maze task. During the initial testing phase, rats were given four trials/day for four consecutive days. They had a maximum of 60 seconds to locate the hidden platform. This figure represents a collapsed analysis of latency measures for each group across the four days of testing. On the first day of testing, the P14 Flight group took longer to locate the hidden platform. However, statistical analyses revealed no significant differences among the FLT, VIV, and AGC groups within each age group (P8 or P14).

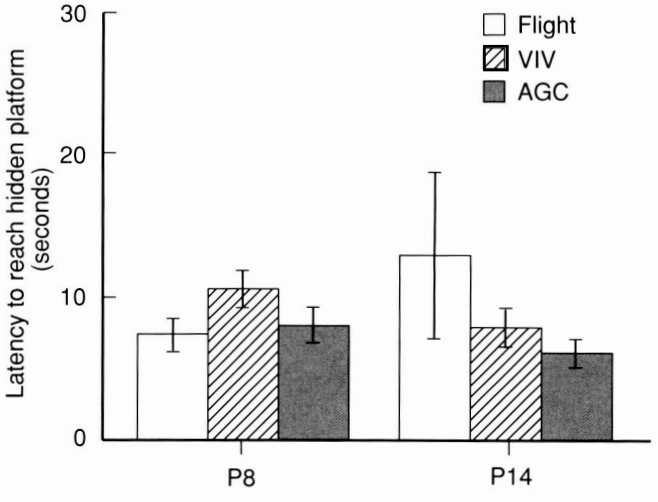


Figure 2. On R+25 postlanding, rats were tested again in the Morris water maze for retention of the task (i.e., memory of the location of the hidden platform). Rats were tested one day (four trials) with a maximum of 60 seconds to locate the hidden platform. This figure represents the average latency for each group during the one day of retesting (i.e., four trials). Analysis of the latency measure for each litter indicated no significant differences among the FLT, VIV, and AGC groups within each age group (P8 or P14).

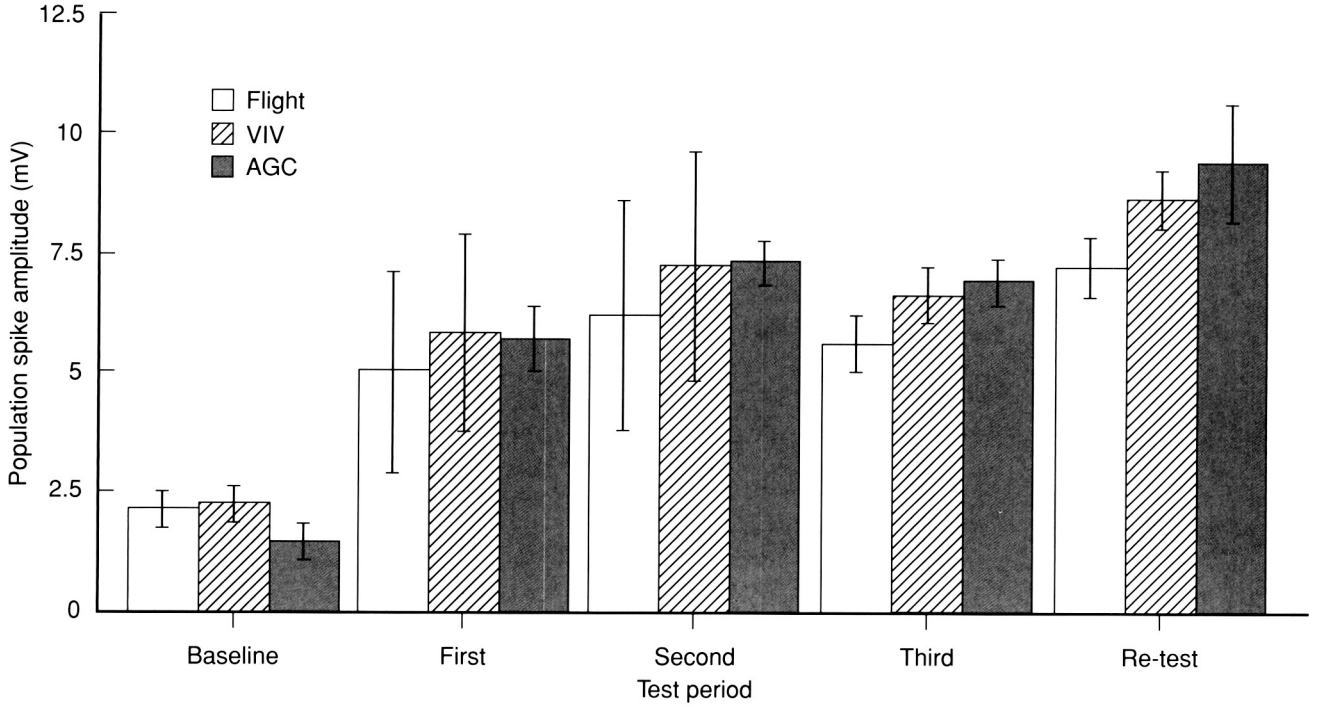
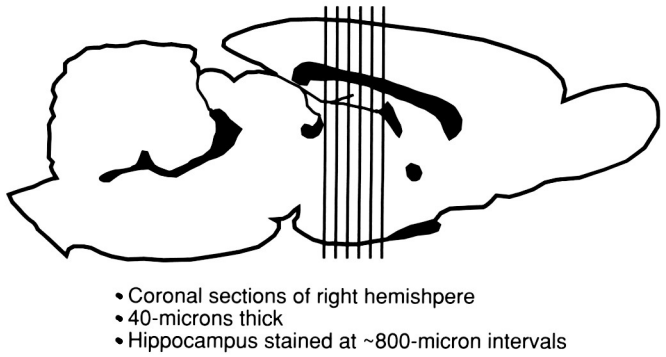


Figure 3. Electrophysiology studies were conducted in a group of P8 FLT, VIV, and AGC animals at R+ 30 postlanding. The bar graph illustrates the degree of LTP (long-term potentiation) induced by high-frequency stimulation of the perforant path projections to the dentate gyrus. Baseline = average response amplitude prior to the delivery of high-frequency stimulation. The three sets of bars indicate average response after separate bouts of stimulation (each bout consisting of 10 trains of eight pulses at 400 Hz delivered once every 10 seconds). Three bouts of stimulation were delivered to induce LTP, and then 10 test pulses were delivered to assess response amplitude. After the final bout, response amplitude was tested for two hours. Then, a final bout of stimulation was delivered to determine the degree of saturation of response amplitude (final set of bars). There was no significant difference among groups in the extent of the initial change in response amplitude or in the duration of LTP.



- Coronal sections of right hemishpere
- 40-microns thick
- Hippocampus stained at ~800-micron intervals

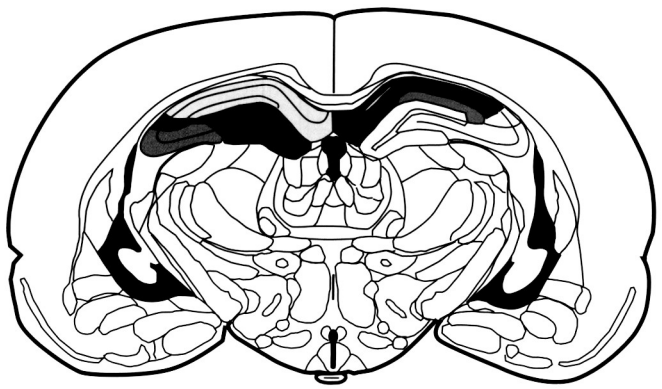


Figure 4. Orientation of hippocampal sections.

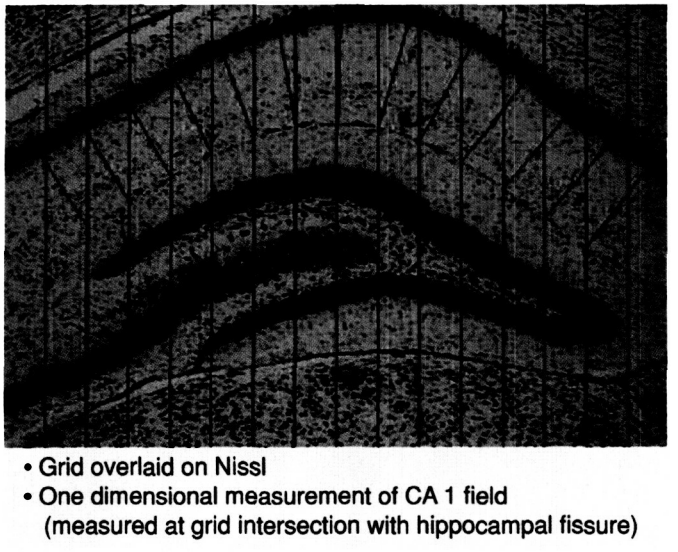


Figure 5. Grid for measurement of intra-hippocampal distances to estimate changes in CA1.

there may be differences if the rats are younger at the time of spaceflight and/or if the rats are tested just after returning from a microgravity environment.

Flight rats do not differ from controls in hippocampal anatomy or by antibody detection

Corresponding to the absence of behavioral and electrophysiological findings after spaceflight was the absence of any detectable changes in protein staining (Figure 4-9). The hippocampus appeared to contain a normal number of neurons, normal appearing dendritic arbors, normal axons, normal synapses, and normal receptors. This was true of the group of rats that was launched when eight days old and evaluated immediately upon returning to Earth.

DISCUSSION

Taken together, our findings suggest that development in an environment lacking gravity does not produce any permanent changes in an animal's ability to use spatial information and form memories of place within its environment. The very subtle behavioral differences observed in the P14 FLT litter in the first few days after reentry were transient. There were also no detectable differences in hippocampal anatomy, biochemistry, and a key measure of synaptic function (the capacity for LTP).

Because of sharing paradigms among investigators, we were unable to test both age groups in all three sets of experiments (behavior, electrophysiology, and hippocampal anatomy/antibody detection). In addition, there is no way to distinguish the experience of microgravity from the experience of launch/landing, which produces rapid changes in pressure gradients. We consider that launch/landing and microgravity are both components of any spaceflight experience.

The NASA Neurolab mission provided an unprecedented opportunity to study how spaceflight and microgravity impact development. This is the first study to evaluate brain development and cognitive function of animals that have spent much of their early life in space. Our data strongly suggest that early experience in an environment lacking gravity does not appear to affect adversely the development of an important aspect of the neural learning and memory system. Given the sensitivity of neural systems to early environment, it is quite surprising that the development of the neural systems that mediate spatial cognitive functions are so impervious to what is certainly an extremely unusual experience during the critical period of development—when animals first explore their environment. Apparently, this key memory system can rapidly re-adapt.

These findings bear importantly on the question of how prolonged spaceflight might affect the brain. Our results indicate that the systems that are critical for spatial cognitive function can readily re-adapt to a gravitational field even when most

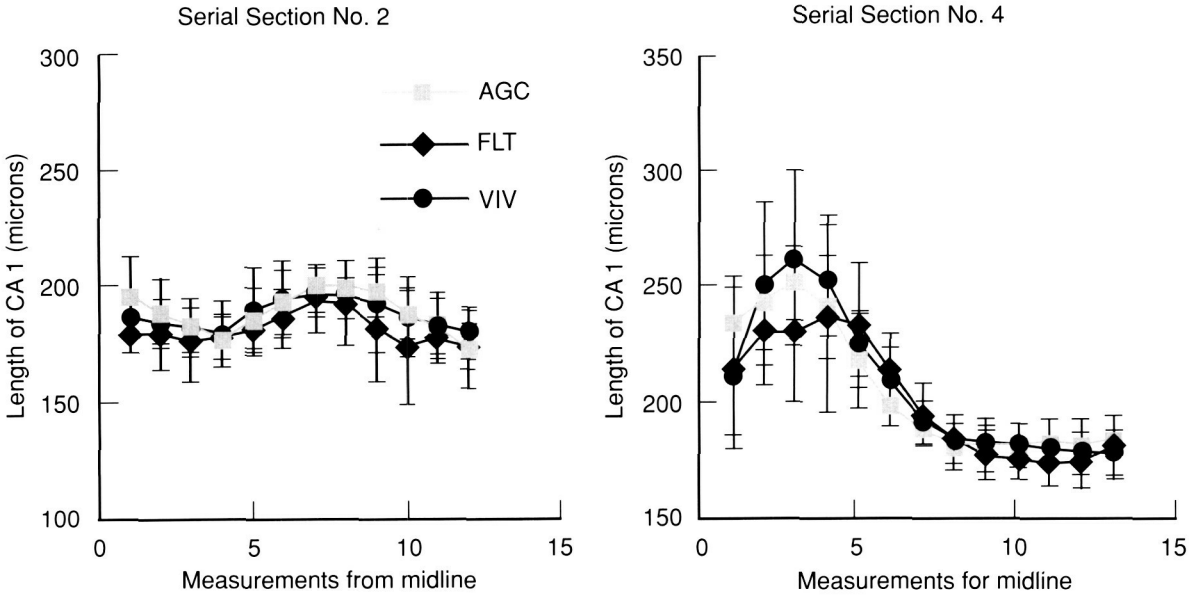


Figure 6. Hippocampal morphometry by Nissl stain reveals no differences in flight rats.

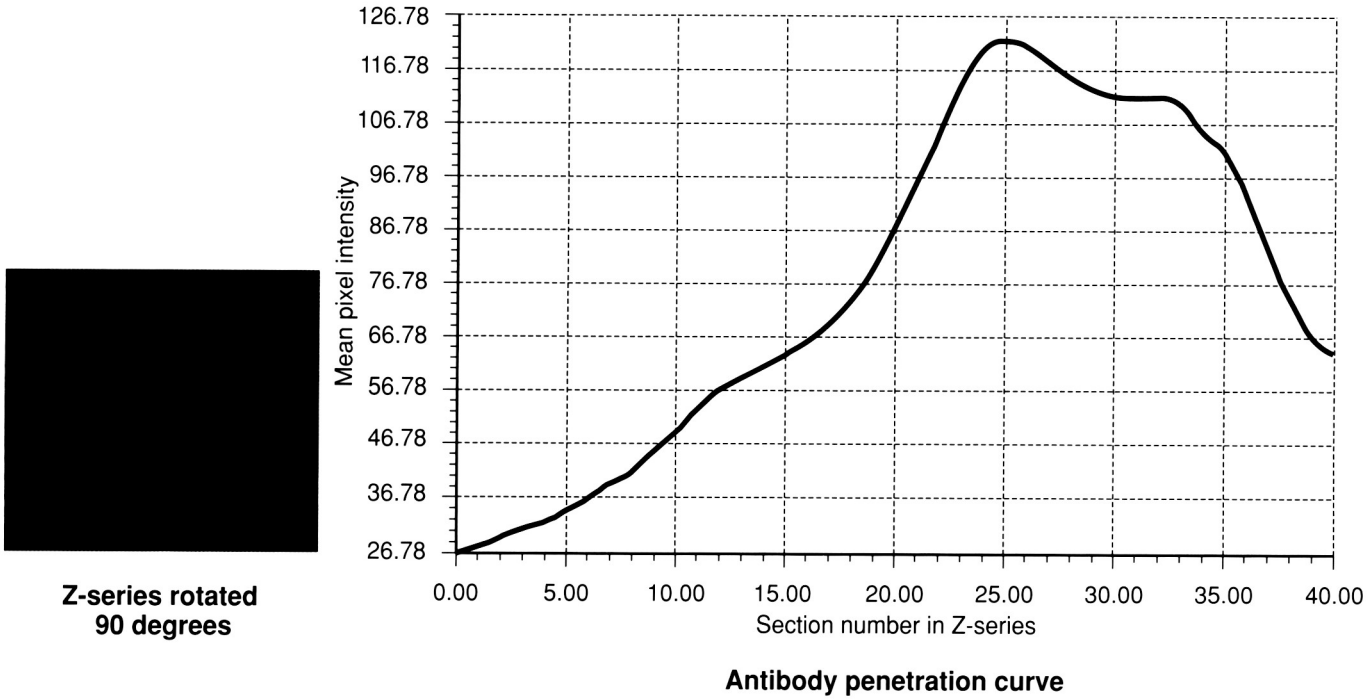


Figure 7. Penetration of the representative (synaptophysin) antibody in the Z-axis.

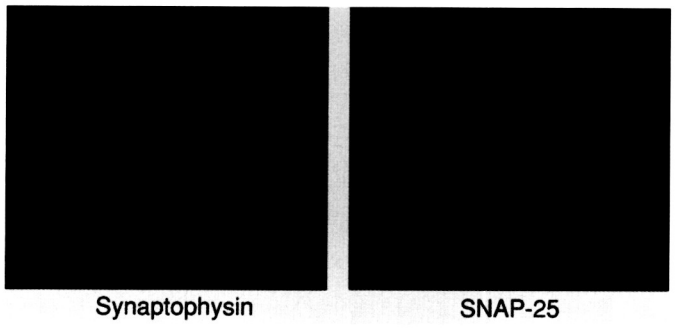


Figure 8. Diffuse antibody labeling of hippocampus from flight rat. For "diffusely" staining antibodies (i.e., receptor and synaptic antibodies), the average pixel intensity of each frame of each Z-series was measured to determine the optimal level of fluorescent expression. The five frames at the optimal level were then averaged into one frame, and the mean pixel intensity for the various regions was measured.

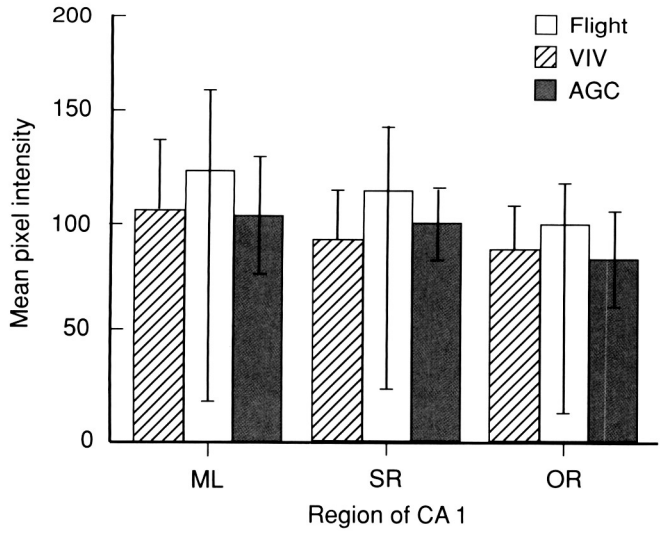


Figure 9. Quantification of the synaptophysin label in flight vs. control rats. No differences were found between the flight and the control rats.

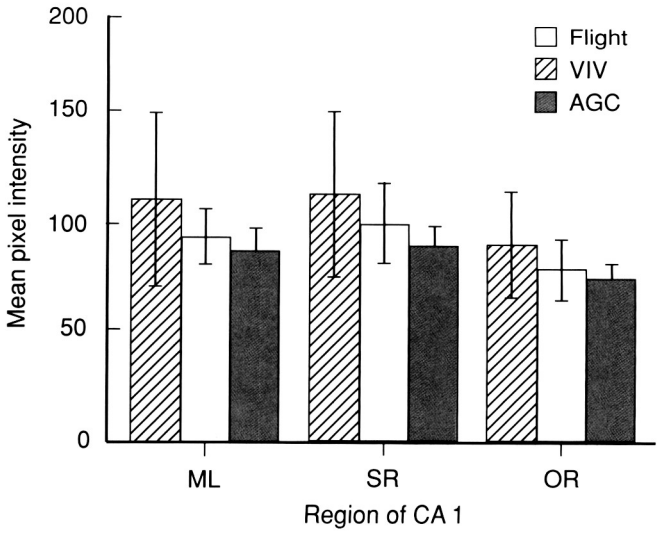


Figure 10. Quantification of the SNAP-25 label in flight vs. control animals. No differences were found between the flight and the control rats.

early development is spent in a gravity-free environment. It is indeed crucial to know that it will be possible to live in space for prolonged periods and even grow up in space without irreversibly altering the memory systems that mediate our basic ability to navigate within the Earth environment.

Acknowledgements

Supported by NASA grant NAG2-964. The authors gratefully acknowledge all of those individuals involved in the NASA Neurolab mission, particularly our team's Experimental Support Scientists (ESSs) Erica Wagner and Tracie Hughes. Special thanks also to the crew of STS-90 for their extraordinary efforts to make the animal experiments a success. We also thank Justin Harder for his technical assistance and Dr. Leonard Jarrard and Dr. Robert Hamm for their advice in designing the behavioral testing paradigm.

REFERENCES

Studies of Brain Weight and RNA Content After Short Periods of Exposure to Environmental Complexity. P. A. Ferchmin, V. A. Eterovic, and R. Caputto. *Brain Res.*, Vol. 20, pages 49–57; 1970.

The Hippocampus as a Spatial Map: Preliminary Evidence From Unit Activity in the Freely-Moving Rat. J. O'Keefe and J. Dostrovsky. *Brain Res.*, Vol. 34, pages 171–175; 1971.

The Hippocampus as a Cognitive Map. J. O'Keefe and L. Nadel. Clarendon Press, Oxford, 1978.

A Review of the Hippocampal Place Cells. J. O'Keefe. *Prog. Neurobiol.,* Vol. 13, pages 419–439; 1979.

On the Trail of the Hippocampal Engram. J. O'Keefe and D. H. Conway. *Physiol. Psychol.*, Vol. 8, pages 229–238; 1980.

Effects of Environmental Complexity and Deprivation on Brain Anatomy and Histology: A Review. R.N. Walsh. *Int. J. Neurosci.*, Vol. 12, pages 33–51; 1981.

Effects of Complex or Isolated Environments on Cortical Dendrites of Middle-Aged Rats. E.J. Green, W.T. Greenough, and B.E. Schlumpf. *Brain Res.,* Vol. 264, pages 233–240; 1983.

Brief Exposure to an Enriched Environment Improves Performance on the Morris Water Task and Increased Hippocampal Cytosolic Protein Kinase C Activity in Young Rats. R. Paylor, S.K. Morrison, J.W. Rudy, L. T. Waltrip, and J.M. Wehner. *Behav. Brain Res.,* Vol. 52, pages 49–59; 1992.

Three-Dimensional Spatial Selectivity of Hippocampal Neurons During Space Flight. J.J. Knierim, B.L. McNaughton, and G.R. Poe. *Nat. Neurosci.*, Vol. 3, pages 209–210; 2000.

Spatial Learning and Memory Is Preserved in Rats after Early Development in a Microgravity Environment. M. D. Temple, K. S. Kosik, and O. Steward. *Neurobiol. Learning Mem.*, Vol. 78, pages 199-216; 2002.